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Evaluation of antagonistic effects of ethanol leaf extract of *Azadirachta indica* on tuber rot pathogens of yam tuber

Abstract

One of the main constraints to yam availability in Nigeria is losses in storage due to fungal rot. The use of plant extracts for controlling yam rots had rather proved effective. Therefore, this study was conducted to investigate the effects of ethanol extracts of *Azadirachta indica* (*A. Juss*), on four isolated rot fungi: *Aspergillus flavus, Aspergillus glaucus, Aspergillus niger* and *Botryodiplodia theobromae*. Rot fungi were isolated from infected yam tubers and cultured on potato dextrose agar (PDA). Pathogenicity test was carried out by inoculating the fungal isolates into healthy yam tubers and these were observed for rot symptom while ethanol at varied concentrations were used as extractants on dried and pulverised leaves of the test plant. The effects of the extracts on the fungi were determined using poisoned food method. All the extracts were inhibitive at varied degrees as they significantly inhibited the mycelia growths of the pathogens both *in vivo* and *in vitro* with varied percentage inhibition, Both 10% and 50% ethanol extract of *A. indica* at 50g/100ml inhibited *A. niger* to 97.67% and 99.17% respectively. All the effects of the extracts on the rot pathogens were significantly different from the effects of the standard and control.

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Introduction

A. indica (*A. juss*) belongs to the family Meliaceae, commonly called neem; it is an important medicinal plant.[1] The leaves, barks, woods, roots and fruits are intensely bitter. Aqueous and ethanol extracts of *A. indica* are widely used as tonic, stimulant and against various ailments.[2] Extracts of *A. indica* fruit, seed, seed kernel, twig, stem bark, and root bark have been reported to possess anti-tumour, anti-inflammatory, immune-stimulating activities, antipyretic, analgesic, diuretic, hypoglycaemic, cardiovascular, anti-microbial, anti-viral, anti-thematic and anti-malaria agents.[3] Residual effect of extracts of leaves was reported to be active against poliomyelitis, herpes, virus, small pox, chickenpox, polio virus, etc.[4]

The use of leaf extract of *A. indica* has proved effective in protecting both pre and post harvest yams tubers from diseases due to its antifungal properties. Extracts from different parts of *A. indica* have been reported to be active against several diseases and vectors that infect human being and animals.[5] According to Ayurveda (Hindu system of medicine), different parts of this tree possess different medicinal properties. A tea prepared from the leaves is used as a favorite wash to cure old ulcers while the aqueous extract of the leaves in particular is used for curing malaria, intestine disorders, and skin problems and even as tooth brushes.[6] The methanol extract of *A. indica* exerts a pronounced anti-inflammatory effect (rat pan edema) a fairly good antipyretic effect (Progeny induced hyperpyrexia) in rabbit and this may justify its use for the treatment of malaria fever.

Materials and method

Collection of infected and healthy tubers from Oja-Oba market in Ado-Ekiti. Five samples were collected from each selling point, placed in sterile polyethylene bags and conveyed into the laboratory for fungal isolation and identification. The identified isolates were used to infect healthy tuber to establish their pathogen city.

Isolation of the fungal organisms

Diseased parts of the yam tubers were cut aseptically into small bit using sterile knife. The diseased bits were sterilized in 70% ethanol, plated in solidified potato dextrose agar (PDA) and were incubated in the dark for 72hours at room temperature (28+2°C). The incubated fungal colonies were sub-cultured into fresh medium to obtain pure culture. Microscopic examination of colony characteristics and morphologies were examined. The morphology and culture characteristics observed were compared with structures in Snowdon.

Pathogen city test

Healthy tubers were surface sterilized with 0.1M mercuric chloride ($HgCl_2$) for a minute and washed in five changes of distilled water. A 5ml cork borer was punched to a depth of 4mm into the healthy tubers and the bored tissues were removed. Five mm diameter disc from the pure culture was placed back. The hole was sealed with candle wax according to the method of Fawole and Oso.[7] The control was set up in the same manner but sterile agar disc replaced fungal inoculum.

Preparation of plant extracts

Leaves of *A. indica* was air-dried and pulverised; thirty grams of the sample was added to15ml of distilled water in flasks. This was vigorously homogenised and left to stand for 24hours. The sample was filtered with a What man paper (No1) and the filtrate used as extract.

Effect of plant extracts on fungal growth

Varied percentage of extract was poured into separate flat bottom flask containing sterilized potato dextrose broth, with a sterile cork borer; the different isolated rot fungi were inoculated into separate flask and incubated at room temperature (28+2°C) for 7 days. No plant extract was added to media for the control.

Effects of extracts on mycelial extension

The method of Amadioha & Obi[8] was used to determine the effect of extracts on mycelial extension of the fungi obtained by placing one disc (3mm diameter) of 5 days old culture of the pathogens in each of five Petri-dishes (1cm diameter) with 170ml PDA medium and 3ml leaf extract. The control experiments were setup with 3ml of sterile distilled water. Five replications of leaf extracts agar per isolate were incubated at room temperatures (28+2°C) for 7 days. Daily measurements of the mycelial extension of the cultures were determined by measuring culture along two diameters, mycelial growth inhibition was taken as growth of the fungus on the leaf extract agar expressed as percentage of growth on the PDA. Fungi toxicity was determined in form of percentage growth of colony inhibition and calculated according to mycelia inhibition.

$$(\%)\ formula = \left(\frac{DC-DT}{DC} x \frac{100}{1}\right)$$

Where DC =Average diameter of colony with control.

DT=Average diameter of colony with treatment.

Results

The fungitoxic potentials of 10, 30 and 50% ethanol leaf extract of *A. indica* on the fungal rot organisms differed significantly ($p<0.05$) from the untreated control and standard. The bio-toxicity of 10, 30 and 50% ethanol leaf extracts of *A. indica* on the rot organisms increased with the increase in concentration.

Effects of 10% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

The bio-protective effects of 10% ethanol leaf extracts of *A. indica* on the fungal pathogens are presented in Table 1. Antifungal effects of 10% ethanol leaf extracts of *A. indica* at 10-50g/100ml on *B. theobromae* ranged from 88.95% to 95.14%. The reduction effect on *B. theobromae* by 10% ethanol leaf extract of *A. indica* at 50g/100ml was 95.14%, followed by antifungal effects of 94.86%, 91.14% and 90.95% against *B. theobromae* by 10% ethanol leaf extract of *A. indica* at 40g, 30 and 20g/100ml respectively. The least anti-phytopathogenic activity of 10% ethanol leaf extract of *A. indica* at 10g/100ml was elicited on *B. theobromae* (88.95%).

Table 1 Effects of 10% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

	% inhibition of mycelial growth			
g/100ml of 10% Ethanol)	***B. theobromae***	***A. flavus***	***A. glaucus***	***A. niger***
10	88.95^{b}	90.75^{a}	88.56^{b}	93.67^{a}
20	90.95^{ab}	91.30^{a}	90.56^{b}	95.92^{a}
30	91.14^{ab}	92.09^{a}	92.11^{ab}	96.42^{a}
40	94.86^{a}	94.09^{a}	92.11^{ab}	97.17^{a}
50	95.14^{a}	94.87^{a}	95.22^{a}	97.67^{a}
Standard	40.70^{c}	30.20^{b}	60.50^{c}	50.00^{b}
Control	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{c}

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan multiple range test.

Effects of 10% ethanol leaf extracts of *A. indica* at 10-50g/100ml were on *A. flavus* ranging from 90.75% to 94.87%. The effect of 10% ethanol leaf extracts of *A. indica* at 50g/100ml was on *A. flavus* (94.87%), while 10% ethanol leaf extracts of *A. indica* at 40g and 30g/100ml, causing 94.09% and 92.09% inhibition on *A. flavus* respectively. *A. flavus* (90.75%) was inhibited by10% ethanol leaf extracts of *A. indica* at 10g/100ml. Biocidal effects of 10% ethanol leaf extract of *A. Indica* at 10-50g/100ml on *A. glaucus* ranged from 88.56% to 95.22%. Inhibition of 10% ethanol leaf extracts of *A. indica* at 50g/100ml on *A. glaucus* (95.22%), followed by effects of 92.11% on *A. glaucus* by 10% ethanol leaf extracts of *A. indica* by both 40g and 30g/100ml respectively. Mycelial growth of 10% ethanol leaf extracts of *A. indica* at 20g/100ml was on *A. glaucus* by 90.56%.

Antifungal effects of 10% ethanol leaf extract of *A. indica* at 10-50g/100ml on *A. niger* ranged between 93.67% and 97.67%. The phytotoxic capacities of 10% ethanol leaf extract of *A. indica* at 50g/100ml was on *A. niger* (97.67%), followed by 10% ethanol leaf extract of *A. indica* at 40g, 30g and 20g/100ml, exercising antimycotic effects of 97.17%, 96.42% and 95.92% against *A. niger* respectively. Also, 10% ethanol leaf extract of at 10g/100ml was antifungal on *A. niger* (93.67%) (Table 1).

Effects of 30% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

The bio-active effects of 30% ethanol leaf extracts of *A. indica* on the fungal pathogens are presented in Table 2. The fungi toxic potentials of 30% ethanol leaf extract of *A. indica* at 10-50g/100ml on *B. theobromae* ranged between 73.71% and 96.57%. The most antimycotic effects of both 50g and 40g/100ml were exhibited against *B. theobromae* (96.57%) by 30% ethanol leaf extract of *A. indica*, followed by growth retardation effects of 94.76% and 92.57% on *B. theobromae* by 30% ethanol leaf extract of *A. indica* at 30g and 20g/100ml respectively, while the lowest effect was elicited by 30% ethanol leaf extract of *A. indica* at 10g/100ml on *B. theobromae* (73.71%).

Antimicrobial effects of 30% ethanol leaf extract of *A. indica* at 10-50g/100ml on *A. flavus* ranged from 96.32% to 100%. Inhibitory effects of 30% ethanol leaf extract of *A. indica* at 30-50g/100ml, caused 100% on *A. flavus*, *A. flavus* (96.32%) was most resistant to 30% ethanol leaf extract of *A. indica* at 10g/100ml.

Phytotoxic effects of 30% ethanol leaf extract of *A. indica* at 10-50g/100ml were high on the degrading organisms ranging from 56.89% to 96.00%. The highest fungicidal effect of 96.00% was against *A. glaucus* by 30% ethanol leaf extract of *A. indica* at 50g/100ml, followed by inducing antimicrobial capacities of 94.56% and 93.33% against *A. glaucus* by 30% ethanol leaf extracts of *A. indica* at 40g and 30g/100ml respectively, mycelial growth of *A. glaucus* (91.55%) was reduced by 30% ethanol leaf extract of *A. indica* at 20g/100ml, while the least effect of 56.89% was by 30% ethanol leaf extract of *A. indica* 10g/100ml against *A. glaucus*.

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Antifungal indications of 30% ethanol leaf extracts of *A. indica* at 10-50g/100ml on the rot microbes ranged from 68.08% to 97.72%. Antimycotic effect of 30% ethanol leaf extract of *A. indica* at 50g/100ml had 97.72% on *A. niger*, followed by inhibitory effects of 96.00% and 71.60% against *A. niger* by 30% ethanol leaf extracts of *A. indica* at 40g and 30g/100ml (Table 2).

Table 2 Effects of 30% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

	% inhibition of mycelial growth			
g/100ml of 30% Ethanol)	***B. theobromae***	***A. flavus***	***A. glaucus***	***A. niger***
10	73.71[b]	96.32[a]	56.89[b]	68.08[b]
20	92.57[a]	97.43[a]	91.55[a]	68.33[b]
30	94.76[a]	100.00[a]	93.33[a]	71.60[b]
40	96.57[a]	100.00[a]	94.56[a]	96.00[a]
50	96.57[a]	100.00[a]	96.00[a]	97.72[a]
Standard	40.70[c]	30.20[b]	60.50[b]	50.00[c]
Control	0.00[d]	0.00[c]	0.00[c]	0.00[d]

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan's Multiple Range Test (DMRT).

Effects of 50% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

The antimycelial effects of 50% ethanol leaf extracts of *A. indica* on the fungal pathogens are presented in Table 3. Antifungal effects of 50% ethanol leaf extracts of *A. indica* at 10-50g/100ml on rot microbes ranged from 94.95% to 97.05%. The highest mycelial reduction effect of 50% ethanol leaf extracts of *A. indica* at 50g/100ml was displayed against *B. theobromae,* eliciting 97.05%, followed by antifungal effects of 96.86% and 95.43% against *B. theobromae* as induced by 40g and 30g/100ml of 50% ethanol leaf extracts of *A. indica,* in addition, 50% ethanol leaf extracts of *A. indica* at 20g/100ml, elicited 95.14% against *B. theobromae.* The least anti-phytopathogenic potential was from 50% ethanol leaf extracts of *A. indica* at 10g/100ml was on *A. glaucus* (94.95%).

Table 3 Effects of 50% ethanol leaf extracts of *A. indica* on mycelial growth of fungal rot organisms

	% inhibition of mycelial growth			
g/100ml of 30% Ethanol)	***B. theobromae***	***A. flavus***	***A. glaucus***	***A. niger***
10	94.95[a]	95.99[a]	92.78[a]	95.58[a]
20	95.14[a]	96.21[a]	93.22[a]	95.75[a]
30	95.43[a]	97.99[a]	94.89[a]	96.33[a]
40	96.86[a]	98.00[a]	95.78[a]	98.69[a]
50	97.05[a]	98.33[a]	96.56[a]	99.17[a]
Standard	40.70[b]	30.20[b]	60.50[b]	50.00[b]
Control	0.00[c]	0.00[c]	0.00[c]	0.00[c]

Mean with the same letter(s) within a column are not significantly different ($p<0.05$) according to the Duncan multiple range test.

Antimycotic effects of 50% ethanol leaf extracts of *A. indica* at 10-50g/100ml on *A. flavus* ranged between 95.99% and 98.33%. The biocidal indication of 50% ethanol leaf extracts of *A. indica* at 50g/100ml was most on *A. flavus*, inducing 98.33%, followed by 98.00%, 97.99% and 96.21% inhibitions on *A. flavus* by 50% ethanol leaf extracts of *A. indica* at 40g, 30g and 20g/100ml respectively. The least effective concentration of 50% ethanol leaf extracts of *A. indica* at 10g/100ml was on *A. flavus* by 95.99%.

Biocidal effects of 50% ethanol leaf extracts of *A. indica* at 10-50g/100ml on *A. glaucus* ranged between 94.89% and 97.99%. Inhibition by 50% ethanol leaf extracts of *A. indica* at 50g/100ml was 97.99% inhibition on *A. glaucus,* followed by 50% ethanol leaf extracts of *A. indica* at 40g and 30g/100ml, exhibiting 95.78% and 94.89% bio-activity on *A. glaucus* respectively. Fungicidal effect of 50% ethanol leaf extracts of *A. indica* at 20g/100ml was on *A. glaucus* by 93.22%. *A. glaucus* (94.89%) exhibited highest resistance to antiparasitic effect of 50% ethanol leaf extracts of *A. indica* extract at10g/100ml.

Antimicrobial effects of 50% ethanol leaf extracts of *A. indica* at 10-50g/100ml on *A. niger* ranged from 95.58% to 99.17%. The greatest phytotoxic effects of 50% ethanol leaf extracts of *A. indica* at 50g/100ml was against *A. niger* (99.17%), followed by inducing 98.69%, 96.33% and 95.75% by 50% ethanol leaf extracts of *A. indica* at 40g, 30g and 20g/100ml against *A. niger* respectively, while the least fungitoxic effect of 95.58% was manifested on *A. niger* by 50% ethanol leaf extracts of *A. indica* at 20g/100ml (Table 3).

Discussion

The radial growth of all the rot fungi: *A. niger, A. flavus, A. glaucus* and *B. theobromae* were significantly inhibited by *A. indica.*

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The antifungal effects of leaves extracts of *A. indica* corroborated the report of,[9] that ethanol and methanol extracts in different concentrations (25%, 50%, 75% and 100%) retarded the growth of the fungal pathogenic isolates: *Aspergillus flavus, Alternaria solani* and *Cladosporium* sp, using disc diffusion method. Similarly, the antimycotic effects of *A. indica* on the rot organisms in this study corroborated the finding of,[10] who evaluated the effects of neem, 3% sodium hypochlorite and 2% chlorohexadine on *C. albican,* an *in vitro* study. Neem significantly reduced the incidence of *C. albican* more than 3% sodium hypochlorite and 2% chlorohexadine. Moreover, the antidermatophytic effects of leaves extract of neem on some human pathogens namely: *Trichophyton mentagrophytes*, *Trichophyton rubrum, Microsporum gypseum* and *Trichophyton tonsurans* using ethanol, ethyl acetate and hexane as extractants have been reported by Radhika et al.[11] Obilo et al.,[12] reported that the wood ash from *A. indica* proved active in the control of *B. theobromae* responsible for most yam tuber rots. Obilo et al.,[12] also reported that wood ash paste from neem tree was able to prevent yam tubers from infection throughout twenty–four months in storage. The Biological control is generally favoured as a method of plant diseases management because it does not have demerit of petrochemicals.[8] The pesticides of botanical origin are non–hazardous, easily to formulate and applied with little or no literacy of the rural dwellers.

Acknowledgment

None.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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